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ORIGINAL ARTICLE

Synthesis, characterization, single crystal XRD, *in vitro* antimicrobial and cytotoxicity study of tris(ethylenediamine)cobalt(III)chloride oxalate trihydrate

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KEYWORDS

Cobalt complex;
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Abstract The complex tris(ethylenediamine)cobalt(III)chloride oxalate trihydrate $[\text{Co}(\text{en})_3]\text{Cl}(\text{C}_2\text{O}_4)\cdot 3\text{H}_2\text{O}$ crystallizes in the monoclinic space group C_2/c with the following unit cell parameters $a = 19.9318$ (13), $b = 9.3344$ (4), $c = 19.0881$ (13) Å $\beta = 96.846(3)^\circ$, $Z = 8$. The crystal structure was solved by direct methods and refined by full matrix least squares procedures to a final R value of 0.0314 for 4330 observed reflections. The reported cobalt complex is six co-ordinated through amine nitrogen with distorted octahedral geometry. There are uncoordinated chloride and oxalate ions along with the water molecules. *In-vitro* antimicrobial activity was studied against various test organisms and found to be good. From *in-vitro* cytotoxic activity of the synthesized complex, the IC_{50} value was found to be 55.85 µg/ml.

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1. Introduction

Metals have been used in the treatment of diseases of humans since ancient times. The Chinese were using elemental gold for the treatment of diseases, a practice known as chrysotherapy, as far back as 2500 BC (Merchant, 1998). Only a small number

of cobalt(III) complexes are known to have biochemical roles. Vitamin B12 is a cobaloxime, a cobalt complex containing a glyoxime ligand, and is one of the rare examples of a naturally occurring organometallic complex *i.e.* possessing a metal carbon bond. A large number of reports on the antibacterial properties of cobalt complexes have appeared in the literature, with Co(II) complexes being the most studied, presumably due to their aqueous stability, availability, and ease of synthesis. However a number of examples of stable Co(III) complexes have also been reported. Reports on the antibacterial properties of cobalt(III) complexes frequently emphasize the increased effectiveness of cobalt ion coordination to a particular ligand when compared to the free ligand itself. Chelation of a bulky ligand to a metal cation reduces the polarity of the ion due to ligand

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orbital overlap with the metal orbitals resulting in a delocalization of positive charge. An increase in lipophilicity of a metal complex enhances bacterial cell membrane penetration and blocking of metal binding sites on enzymes. Nagababu and co-workers screened a large number of bis(ethylenediamine)cobalt(III) cations against gram-positive and gram-negative bacteria *Escherichia coli*, *Salmonella typhimurium*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis* (Nagababu et al., 2006).

Cobalt complexes have attracted a great deal of attention amongst the scientific community due to their therapeutic uses as tumour imaging agent, antitumour, transport protein transferrin (Tf), antiviral, antiparasitic, antithrombotic, enzymatic therapeutics, anti-inflammatory activities and as metabolic modifier (Smith, 2005; Liang et al., 2004; Maccari et al., 2004; Unitt et al., 1999; Mishra et al., 2008). The aim of our present work is to co-ordinate the metal ion with ethylenediamine and oxalic acid and study their pharmacological action.

2. Materials and methods

All reagents, ethylenediamine, oxalic acid and cobalt(II)chloride were purchased from Sigma Aldrich, India.

2.1. Physical measurements

Micro analytical data of the compounds were recorded in the *Elementar Vario EL III* CHN analyser. The FT-IR spectra of the samples were recorded on a *Shimadzu* spectrophotometer in 4000–400 cm^{-1} . The UV–Visible spectra were recorded on an *Elico SL 159* UV–Vis spectrophotometer. Magnetic susceptibility measurements of the complexes were carried out by *Guoy balance* using copper sulphate as the calibrant. The molar conductance of the complex was measured using a *sys-tronics* conductivity bridge at room temperature in DMSO solution. The antimicrobial activities of the ligand and its complex were carried out by the disc diffusion method. The thermal behaviour of Co(III) complex was studied using *Perkin Elmer STA 6000* thermoanalyser.

2.2. Synthesis of tris ethylenediamine cobalt(III)chloride oxalate trihydrate

An ethanolic solution of ethylenediamine (0.03 mol, 1.8 ml), oxalic acid (0.01 mol, 0.63 g) and cobaltous chloride (Canpolat et al., 2004) $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.01 mol, 2.37 g) was refluxed for 2 h in the presence of aq. NH_3 on a water bath. On cooling, the orange colour crystals obtained were filtered, washed with ethanol and dried. The crude product was recrystallized by the slow evaporation method using methanol as solvent. After 15 days diamond shaped single crystals were harvested. Yield: 67%. Anal. Calcd for $\text{CoC}_8\text{H}_{30}\text{N}_6\text{O}_7\text{Cl}$; (%): C, 23.03; H, 7.19; N, 20.15. Found (%): C, 23.02; H, 7.19; N, 20.16 (see Scheme 1).

2.3. Determination of X-ray crystal structure of Co(III) complex

X-ray diffraction for the complex tris ethylenediamine cobalt(III)chloride oxalate trihydrate was made on a *Bruker*

APEX CCD-II area detector diffractometer with graphite monochromated *Mo-K α* radiation ($\lambda = 0.71073$). The crystal structure was solved by direct methods. Structure refinements were performed by full matrix least squares procedures using *SHELXL-97* on F^2 . The crystal data and structural refinement parameters are summarized in Table 1.

2.4. Antimicrobial activity

The antibacterial activity of the metal complex was screened for gram positive bacteria and gram negative bacteria namely *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* respectively by the disc diffusion method using agar nutrient as the medium. The antifungal activities were screened for the organisms *Mesodinium rubrum*, *Aspergillus niger* and *Candida albicans* by the disc diffusion method cultured on potato dextrose agar as medium. The plate was incubated 24 h for bacteria and 72 h for fungi. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. An inhibition zone was developed, at which the concentration was noted (Chohan et al., 2005).

2.5. Cytotoxic activity

The cobalt complex was checked with its cytotoxic activity (MTT assay) against MCF-7 which was obtained from National Centre for Cell Science, Pune. The cells were grown and maintained at 37 °C, 5% CO_2 , 95% air and 100% humidity. After 48 h of incubation, MTT was added and incubated at 37 °C for another 4 h. The medium with MTT was flicked off and measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was calculated with respect to the control.

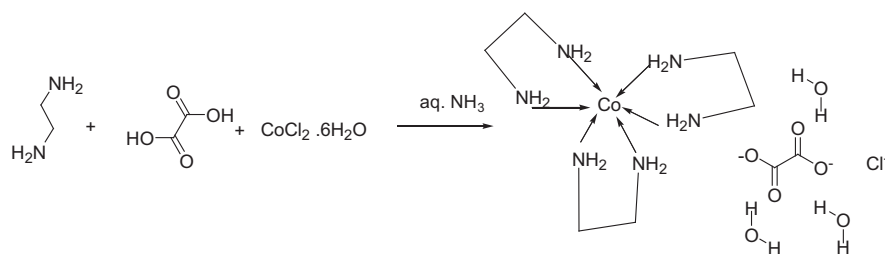
3. Spectral discussion

3.1. General properties of tris(ethylenediamine)cobalt(III)chloride oxalate trihydrate

The analytical data of the complex are in good agreement with the experimental values. The complex is soluble in common polar solvents but readily soluble in DMF and DMSO to give stable solutions at room temperature. In the solid state, the Co(III) complex is fairly stable in air allowing physical measurements. The molar conductance of Co(III) complex is $74 \text{ Smol}^{-1} \text{ cm}^{-1}$ showing their electrolytic nature (Geary, 1971).

3.2. FT-IR spectral studies of the ligand and tris(ethylenediamine)cobalt(III)chloride oxalate trihydrate

The FT-IR spectral data provide valuable information regarding the nature of the functional group attached to the metal atom. In the ethylenediamine cobalt complex, two bands appeared at 3195 and 3074 cm^{-1} which correspond to the $-\text{NH}_2$ group in the complex (Thankamony and Mohanan, 2007; Raman et al., 2004). A band at 1744 cm^{-1} is attributed to the $>\text{C}=\text{O}$ vibration in the oxalate group. From IR, the exact co-ordination site for this complex is amino nitrogen which was further confirmed by single crystal XRD studies. The IR spectrum of the complex is given in Fig. 1.



Scheme 1 Synthesis of tris(ethylenediamine)cobalt(III)chloride oxalate trihydrate.

Table 1 Crystal data and structure refinement for $[\text{Co}(\text{en})_3]\text{Cl}(\text{C}_2\text{O}_4)\cdot 3\text{H}_2\text{O}$.

Empirical formula	$\text{CoC}_8\text{H}_{30}\text{N}_6\text{O}_7\text{Cl}$
Formula weight	416.76
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, C_2/c
Unit cell dimensions	$a = 19.9318(13)$ Å $\alpha = 90^\circ$ $b = 9.3344(4)$ Å $\beta = 96.846(3)^\circ$ $c = 19.0881(13)$ Å $\gamma = 90^\circ$
Volume	$3526.0(4)$ Å ³
Z, Calculated density	8, 1.570 Mg/m ³
Absorption coefficient	1.167 mm^{-1}
$F(000)$	1760
Crystal size	$0.40 \times 0.35 \times 0.30$ mm
Theta range for data collection	2.15 to 28.24°
Limiting indices	$-26 \leq h \leq 26$, $-12 \leq k \leq 11$, $-25 \leq l \leq 19$
Reflections collected/unique	13,098/4330 [$R(\text{int}) = 0.0218$]
Completeness to theta	28.24 99.0%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7209 and 0.6525
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4330/9/281
Goodness-of-fit on F^2	1.110
Final R indices [$I > 2\sigma I$]	$R_1 = 0.0314$, $wR_2 = 0.0886$
R indices (all data)	$R_1 = 0.0394$, $wR_2 = 0.0999$
Extinction coefficient	0.0007(2)
Largest diff. peak and hole	0.797 and -0.381 e Å^{-3}

3.3. Electronic absorption spectra of tris(ethylenediamine)cobalt(III)chloride oxalate trihydrate

The electronic absorption spectrum of the complex was recorded using solvent DMF at 25°C . The electronic spectral data are in relevance with proposed geometry of the complex. The electronic spectra of Co(III) complex display bands are at 285, 350 and 400 nm (Fig. 2). The absorptions around 280–360 nm are ascribed to metal to ligand charge transfer. The absorption at 400 nm may be assigned to ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$. These are similar to those reported for other six co-ordinated Co(III) complexes (Raman et al., 2009; Howlader and Islam, 2007; Lever and Mantovani, 1971; Lever, 1968; Guoy and Ballhausen, 1963; Ababei et al., 2011).

3.4. Thermal behaviour (TGA/DTA) of tris(ethylenediamine)cobalt(III)chloride oxalate trihydrate

The thermogravimetric analysis gives information about the thermal stability of the complex and suggests a general scheme

for thermal decomposition of these chelates. In the present investigation, heating rates were suitably controlled at $10^\circ\text{C min}^{-1}$ under nitrogen atmosphere and the weight loss was measured from the ambient temperature up to 800°C . The thermogram of the Co(III) complex shows two decomposition steps within the temperature range 35 – 500°C corresponds to the loss of water molecule of hydration, chlorine and carbon dioxide gases with a mass loss of 34% (Cald: 32.02%) accompanied by two exothermic peak with $t_{\text{max}} = 129.02$ and 371.89°C on the DTA curve, may be attributed to the removal of co-ordinated water and the non-co-ordinated part of the ligand. The subsequent steps ($> 500^\circ\text{C}$) correspond to the removal of the organic part of the ligands, leaving metal oxide as a residue. The overall weight loss amounts to 65% (Cald: 62.6%). This mass loss corresponds to the pyrolysis of the ligand molecules leaving Co_2O_3 as residue. An exothermic peak with $t_{\text{max}} = 551^\circ\text{C}$ on the DTA curve was observed for this step. The thermogram of the Co(III) complex is depicted in Fig. 3.

3.5. Single crystal XRD data of tris(ethylenediamine)cobalt(III)chloride oxalate trihydrate

A single crystal of the title complex measuring $0.40 \times 0.35 \times 0.30$ mm was picked up for X-ray intensity data collection on a Bruker Apex Area II CCD detector. A total number of 13,098 reflections were collected out of which 4330 were treated as observed ($I > 2\sigma I$). Data were corrected for absorption factor. The final refinement cycle yielded an R factor of 0.0314 [$wR_2 = 0.0886$] for 4330 observed reflections. The CIF for this structure has been deposited at Cambridge Crystal Data Centre (CCDC 929136). The crystallographic data are summarized in Table 1. The bond lengths and bond angles are given in Table 2. An ORTEP view of the complex is shown in Fig. 4.

In the cobalt complex three ethylenediamine molecules are bound to the Co(III) ion through nitrogen with the bond lengths Co(1)–N(1), Co(1)–N(2), Co(1)–N(3), Co(1)–N(4), Co(1)–N(5), Co(1)–N(6) as 1.9650(16), 1.9615(15), 1.9613(15), 1.9655(16), 1.9538(15) and 1.9623(15) Å respectively. These are the new bonds formed while complexation. The bond angles of N(1)–Co(1)–N(2), N(3)–Co(1)–N(4) and N(5)–Co(1)–N(6) are $85.17(7)$, $85.78(7)$ and $85.52(7)^\circ$ respectively. The counter ion in most of the ethylenediamine complexes is chloride ion but in this complex the counter ions are chloride and oxalate which is proved from the bond lengths C(7)–C(8), C(7)–O(2), C(7)–O(3), C(8)–O(4), C(8)–O(5) as 1.563(2), 1.254(2), 1.245(2), 1.252(2), 1.245(2) respectively. A packing view of the complex in the unit cell is shown in Fig. 5.

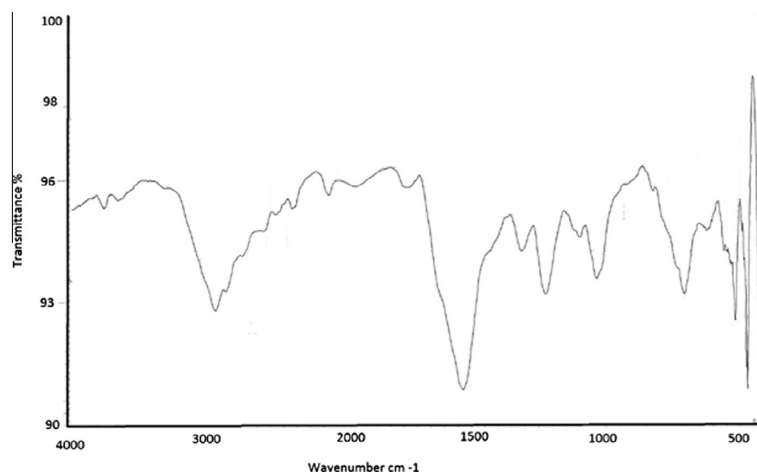


Figure 1 IR Spectrum of $[\text{Co}(\text{en})_3]\text{Cl}(\text{C}_2\text{O}_4)\cdot 3\text{H}_2\text{O}$ complex.

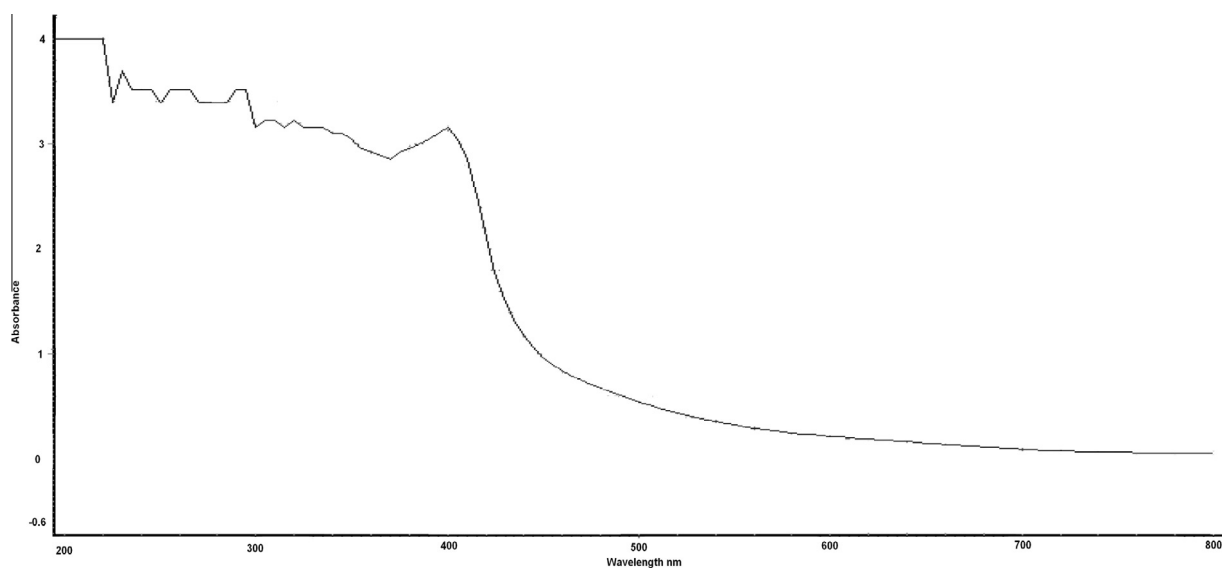


Figure 2 Electronic spectrum of $[\text{Co}(\text{en})_3]\text{Cl}(\text{C}_2\text{O}_4)\cdot 3\text{H}_2\text{O}$ complex.

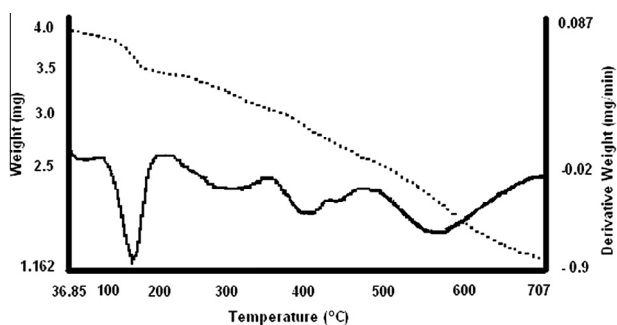


Figure 3 TGA/DTA curve of $[\text{Co}(\text{en})_3]\text{Cl}(\text{C}_2\text{O}_4)\cdot 3\text{H}_2\text{O}$ complex.

Five membered ethylene diamine chelate rings have two possible conformations known as Δ and Λ . When three such chelates form a tris complex, the metal centre is chiral and will have two possible enantiomeric configurations known as Δ and

Λ . Combining all the structural possibility results, even for a simple symmetrical chelate ethylene diamine (en), a total of eight isomers are possible: $\Delta(\delta\delta\delta)$ $\Delta(\delta\delta\lambda)$ $\Delta(\delta\lambda\lambda)$ $\Delta(\lambda\lambda\lambda)$ $\Lambda(\delta\delta\delta)$ $\Lambda(\delta\delta\lambda)$ $\Lambda(\delta\lambda\lambda)$ and $\Lambda(\lambda\lambda\lambda)$. In the present crystal two unusual conformers were found $\Delta(\delta\lambda\lambda)$ and $\Lambda(\delta\delta\lambda)$.

The water of hydration of the above said complex was carried out using the laboratory method and was found to be 2. The crystal structure shows three water molecules. This may be due to a highly disordered water structure being indicative of a dynamic equilibrium between small conglomerates and free molecules.

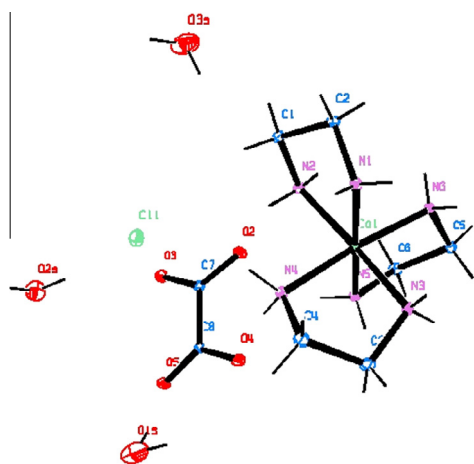
4. Pharmacology

4.1. Antimicrobial activity

The investigated compound was tested against the bacteria *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and fungi *Mesodinium rubrum*,

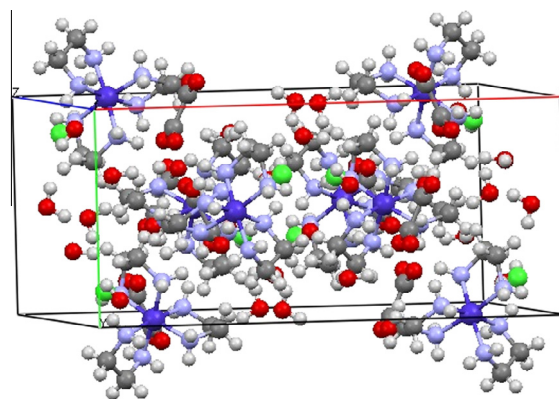
Table 2 Bond lengths [Å] and angles [°] for [Co(en)₃]Cl(C₂O₄)·3H₂O.

Co(1)–N(5)	1.9538(15)	N(5)–Co(1)–N(3)	91.93(7)
Co(1)–N(3)	1.9613(15)	N(5)–Co(1)–N(2)	90.43(7)
Co(1)–N(2)	1.9615(15)	N(3)–Co(1)–N(2)	175.17(7)
Co(1)–N(6)	1.9623(15)	N(5)–Co(1)–N(6)	85.52(7)
Co(1)–N(1)	1.9650(16)	N(3)–Co(1)–N(6)	90.06(7)
Co(1)–N(4)	1.9655(16)	N(2)–Co(1)–N(6)	94.32(7)
O(2)–C(7)	1.254(2)	N(5)–Co(1)–N(1)	174.06(7)
O(3)–C(7)	1.245(2)	N(3)–Co(1)–N(1)	92.75(7)
O(4)–C(8)	1.252(2)	N(2)–Co(1)–N(1)	85.17(7)
O(5)–C(8)	1.245(2)	N(6)–Co(1)–N(1)	90.82(7)
N(1)–C(2)	1.487(2)	N(5)–Co(1)–N(4)	91.14(7)
N(6)–C(5)	1.487(2)	N(3)–Co(1)–N(4)	85.78(7)
N(3)–C(3)	1.482(3)	N(2)–Co(1)–N(4)	89.97(7)
Co(1)–N(2)	1.9615(15)	N(6)–Co(1)–N(4)	174.58(7)
Co(1)–N(6)	1.9623(15)	N(1)–Co(1)–N(4)	92.84(7)
Co(1)–N(1)	1.9650(16)	C(2)–N(1)–Co(1)	109.10(12)
Co(1)–N(4)	1.9655(16)	C(5)–N(6)–Co(1)	109.52(12)
O(2)–C(7)	1.254(2)	C(3)–N(3)–Co(1)	109.73(12)
O(3)–C(7)	1.245(2)	C(6)–N(5)–Co(1)	109.68(12)
O(4)–C(8)	1.252(2)	C(4)–N(4)–Co(1)	109.73(13)
O(5)–C(8)	1.245(2)	C(1)–N(2)–Co(1)	110.53(12)
N(1)–C(2)	1.487(2)	O(5)–C(8)–O(4)	124.63(18)
N(6)–C(5)	1.487(2)	O(4)–C(8)–C(7)	117.25(16)
N(3)–C(3)	1.482(3)	N(6)–C(5)–C(6)	117.93(16)
N(5)–C(6)	1.487(2)	N(3)–C(3)–C(4)	106.97(15)
N(4)–C(4)	1.475(3)	N(1)–C(2)–C(1)	108.67(17)
N(2)–C(1)	1.489(2)	N(5)–C(6)–C(5)	106.82(15)
C(8)–C(7)	1.563(2)	O(3)–C(7)–O(2)	106.95(15)
C(5)–C(6)	1.503(3)	O(3)–C(7)–C(8)	126.50(17)
C(3)–C(4)	1.492(3)	O(2)–C(7)–C(8)	117.09(16)
C(2)–C(1)	1.502(3)	N(2)–C(1)–C(2)	116.30(16)
		N(4)–C(4)–C(3)	107.45(15)

**Figure 4** ORTEP diagram of [Co(en)₃]Cl(C₂O₄)·3H₂O complex.

Aspergillus niger and *Candida albicans*. The minimum inhibitory concentration (MIC) values of the complex against the growth of microorganisms are summarized in Tables 3 and 4.

From Table 3 it is observed that the cobalt complex is more active against all the test organisms especially *Bacillus subtilis* and *Staphylococcus aureus*. This complex serves as a very good anti-fungal agent against *Mesodinium rubrum* and *Candida albicans* and shows a moderate activity against *Aspergillus*

**Figure 5** Packing diagram of [Co(en)₃]Cl(C₂O₄)·3H₂O complex.

niger as shown in Table 4. Such increased activity of the complexes can be explained with respect to Overtone's concept and Tweedy's chelation theory. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid-soluble materials whose liposolubility is an important factor, which controls the antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a great extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of pi electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of micro organisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms (Munde et al., 2012; Pelczar et al., 1996; Mane et al., 2001; Mishra and Singh, 1993; Dharmaraj et al., 2001).

Table 3 Antibacterial activity of the [Co(en)₃]Cl(C₂O₄)·3H₂O complex.

S. No.	Organisms	Zone of Inhibition (mm)	
		Standard	Complex
1	<i>B. Subtilis</i>	21	24
2	<i>S. aureus</i>	11	16
3	<i>E. coli</i>	17	19
4	<i>K. pneumoniae</i>	13	12

Table 4 Antifungal activity of the [Co(en)₃]Cl(C₂O₄)·3H₂O complex.

S. No.	Organisms	Zone of inhibition (mm)	
		Standard	Complex
1	<i>M. rubrum</i>	17	23
2	<i>A. niger</i>	11	12
3	<i>C. albicans</i>	15	26

a) 0.05 μM Concentrationb) 50 μM Concentration

Figure 6 Anticancer activity of $[\text{Co}(\text{en})_3]\text{Cl}(\text{C}_2\text{O}_4)\cdot 3\text{H}_2\text{O}$ complex.

4.2. Cytotoxic activity

To verify this bio-reductive activation mechanism under *in-vitro* conditions, cyto-toxicity studies were carried out. Moreover, as the balance between the therapeutic potential and toxic side effects of a compound is very important when evaluating its usefulness as a pharmacological drug, experiments were designed to investigate the *in-vitro* cytotoxicity of the synthesized cobalt complex against the MCF 7. Cytotoxicity was determined by means of a colorimetric microculture MTT assay, which measures the mitochondrial dehydrogenase activity as an indication of cell viability. It is evident that the number of cells decreased with an increase in the concentration of the Co(III) complex (Fig. 6). The complex showed higher potential antineoplastic activity which is evidenced by low IC_{50} values (50% inhibitory concentration after exposure for 48 h. in MTT assay) of 55.85 $\mu\text{g}/\text{ml}$ (Mosmann, 1983; Monks et al., 1991).

The ability of the cobalt(III) complexes $[\text{Co}(\text{en})_2(\text{pyz})_2]\text{Br}_3$ and $[\text{Co}(\text{en})_2\text{Cl}_2]\text{Cl}$ to induce cytotoxicity was investigated and IC_{50} values for each derivative *i.e.* 110 and 90 $\mu\text{g}/\text{ml}$, respectively were reported by Nagababu and co-workers (Nagababu et al., 2009). In our present investigation, the IC_{50} value of the title complex is 55.85 $\mu\text{g}/\text{ml}$ which is more toxic than the similar cobalt(III) ethylenediamine complexes in the literature.

5. Conclusion

In conclusion, ethylenediamine cobalt complex has been synthesized and the structure has been confirmed by IR, electronic, molar conductance and TGA/DTA studies. The

structure of the complex was further confirmed from single crystal XRD studies. The results of pharmacological evaluation of this complex revealed that it possesses significant *in vitro* anti-microbial and anticancer activity. It is convincing that this complex certainly holds great promise towards the pursuit to discover a novel class of antimicrobial and antitumour agents.

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